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TRANSFERS OF WATER AND SOLUTES IN THE BODY

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PROPERLY KNOWLEDGE of the processes by which water and solutes are transferred in the body begins with the discovery of the circulation of the blood by the great physiological pioneer from whom this society proudly derives its name. This aspect of the subject, however, will receive no attention this evening. The movements of water between the blood stream and the interstitial fluids and the important functions of the lymphatic system will also be neglected, since they have been subjects of recent lectures of Eugene M. Landis[1] and Cecil K. Drinker[2] respectively. I shall confine myself entirely to the discussion of the processes which control the motions of water and solutes between cells and the fluids in which they lie. Even in this connection the effects on these movements of changes in the reactions of the blood and body fluids, which have been so admirably elucidated by Dr. Van Slyke, will receive only scant consideration. Since, as Van Slyke has shown, the effects of pH changes derive from alterations of the osmotic pressure relations between media, they represent only a special case of one

part of the subject of this lecture. History must also largely go by the boards to permit adequate presentation of recent experimental work and its implications. My position here tonight is chiefly that of recorder or reporter of investigations that have been carried out over a period of years in my laboratory by a number of persons whose names will appear as the story unfolds. Relevant material from other sources will be mentioned without any attempt to treat the literature exhaustively.

First I should like to consider certain experiments dealing with the composition of the red blood cell and its behavior in its native extracellular or interstitial fluid, the blood plasma. The red blood cell lends itself to study because it can be isolated for examination and because its metabolic processes are quite slow and can be brought practically to a standstill by moderate chilling. There is much evidence to prove that the contents of the red blood cells have the same osmotic pressure as blood serum and are in osmotic equilibrium with the latter. This means that, in spite of the great differences in their composition, both cells and serum contain the same concentrations of osmotically active chemical components. The differentiation is brought about, apparently, by the fact that the membrane of the cell has a highly selective permeability. It is quite freely permeable to water. If blood is diluted with water this water distributes itself between cells and serum in the same proportion as the water which was originally in these media. The consequence is that the cells swell and, if enough water is added, will ultimately burst. Urea and certain other simple organic compounds also penetrate the cell membrane quite freely. Consequently solutions of urea, although they have a definite osmotic pressure, act exactly like water. Furthermore, urea added to serum does not alter the size or composition of the blood cells. To proteins the cells are quite impermeable, otherwise hemoglobin could escape into the serum. The molecular concentration of protein in the red cells is more than three times as great as that in the plasma. *A priori* it might be expected that the osmotic pressure of the cellular contents would be far greater than that of serum. It is a similar difference in concentration of protein between plasma and interstitial fluids that, according to the Starling theory, balances the hydrostatic force of the blood pressure and retains fluids in the blood stream. In the equilibrium between cells and serum, however, the unequal distribution of protein is compensated by impermeability to other substances, especially the cations, sodium, potassium, calcium and magnesium. If red

blood cells are suspended in solutions of sodium chloride of varying strength it will be found that they retain their original size and shape in that solution which has a concentration of about 0.9 per cent NaCl, or approximately 150 millimolar. Since NaCl in solutions of this strength is almost completely dissociated into Na^+ and Cl^- ions, the solution contains 300 osmotically active milliequivalents. Cells behave in this solution, however, quite differently than they do in a 300 millimolar urea solution. In the latter they swell as they would in so much water. They do not swell in the sodium chloride solution because the sodium is unable to cross the cell membrane. In sodium chloride solutions stronger than 150 millimolar the cells shrink, in weaker solutions they swell. The degree of expansion or contraction depends on the concentration of salt which is added. Equilibrium is reached in each case when enough water has been transferred across the cellular membrane to equalize the concentrations of active osmotic solutes on the two sides of the membrane.

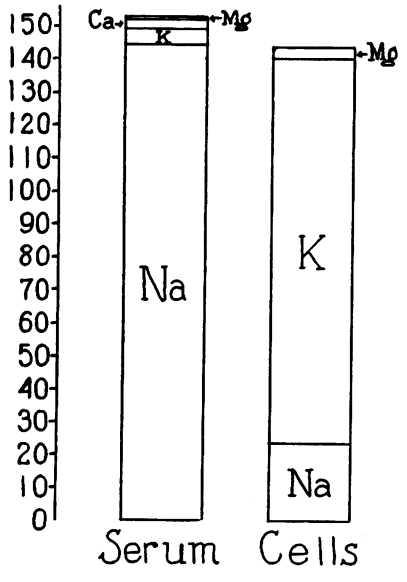

Fig. 1—The average molar concentrations of base in human serum and red blood cells.

After these few preliminary remarks I should like to present certain data and experimental results. Fig. 1 shows the concentration of anions

and cations in normal serum, not as they are usually given, in relation to units of volume, but as osmotic equivalents (millimols) per liter of water. These data are from analyses made by Miss Pauline Hald.[3] The total concentration of base in serum water, 153 millimols, is approximately the same as that of an isotonic salt solution, 150 millimols. For purposes of calculation, then, it should be possible to use the concentration of base in serum as a measure of the effective osmotic pressure of the serum, that is, of the components of serum which cannot cross the cell membrane and which, therefore, will determine the distribution of water between cells and serum. If this is true and if the cell membrane is completely impervious to base, it should follow that, when water or salts or both are added to blood, the concentrations of base in the serum before and after the addition should vary inversely as the volumes of water in the cells.

$$\frac{Bs_1}{Bs_2} \text{ should equal } \frac{Wc_2}{Wc_1}$$

Where B represents mols of base per kilo of water, W kilos of water, the subscripts s and c, serum and cells, and 1 and 2 the bloods before and after treatment, respectively. It will be apparent from the figures in Table I that experimental results conform to expectation with surprising accuracy. In these fourteen experiments, performed by Wakeman, Eisenman and Hald,[4,5] the ratios of base and of cell water are almost identical in the great majority of instances, although water, and the sulfates, carbonates and chlorides of both sodium and potassium were added to blood. Since then similar results have been obtained with phosphates of sodium and potassium. These and other experiments of a like nature can leave no doubt that human red blood cells under the conditions of these experiments are quite impermeable to the bases, sodium, potassium, calcium and magnesium, and that, when the concentrations of base in the serum are changed, the cells give up or take on water in such proportions that the osmotic pressures in the two media always remain identical. Fig. 2, from data of Klinghoffer,[6] shows that solutions of urea (marked by circles) and of glycerol (the triangles) have the same effect as so much water (crosses), proving that these substances enter the cells with perfect freedom. Sucrose is unable to enter the cells; therefore in solutions of this sugar red cells behave as they do in salt solutions.

Glucose presents a rather particular case. It has been demonstrated

TABLE I

Relations of Transfers of Water between Cells and Serum to Changes of Serum Base after Addition to Blood in Vitro of Salts or Water

*Experiment No.**	$\frac{W_{c1}}{W_{c1}}$ (1)	$\frac{B_{s1}}{B_{s2}}$ (2)	(1)/(2)	*Added to blood*
1	0.800	0.798	1.00	34.7 m.eq. NaCl and KCl
4	0.849	0.825	1.03	31.3 " "
5	0.817	0.821	1.00	32.2 " KCl
2	0.928	0.942	0.99	9.2 " Na_2SO_4 and K_2CO_3
3	0.869	0.938	0.93	13.9 " " " "
6	0.906	0.907	1.00	12.9 " Na_2CO_3
7	1.334	1.293	1.03	250 cc. H_2O
8	1.260	1.305	0.97	250 " "
1-*a*	0.934	0.926	1.01	11.5 m.eq. K_2CO_3
2-*a*	0.865	0.757	1.14	35.6 " KCl
3-*a*	0.816	0.779	1.05	35.7 " NaCl
4-*a*	1.189	1.268	0.94	200 cc. H_2O
5-*a*	1.230	1.322	0.93	250 " "
6-*a*	0.871	0.882	0.99	19.7 m.eq. Na_2CO_3
Average ...	.....	.....	1.00	

Experiments 1 to 8 are from Wakeman, Eisenman and Peters;[4] the remainder from Eisenman, Hald and Peters.[5]

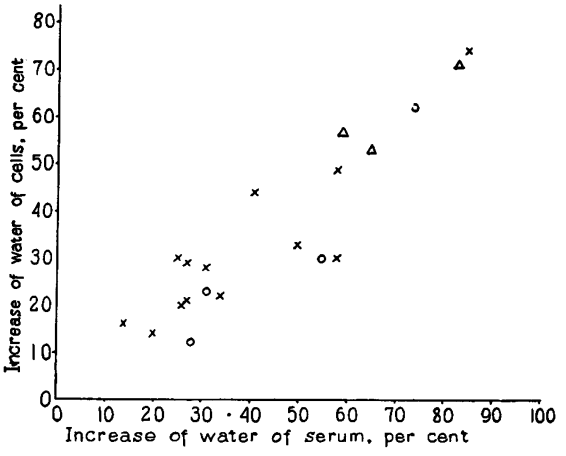

Fig. 2—Per cent increases in water of cells and serum after the addition to blood of water ×, urea solutions o, and glycerol solutions Δ.

repeatedly that in human blood glucose is equally distributed through the water of cells and serum. The addition to blood of dilute solutions of glucose causes cells to swell as they do in water, the glucose and water distributing themselves equally between the two media. In stronger glucose solutions cells do not swell so much, taking up less than their share of glucose. The factors that limit the load of glucose which the cells will assume are not yet clear. Klinghoffer has, however, shown, in some unpublished experiments, that, in spite of this discrepancy in the distribution of the glucose, osmotic equilibrium between cells and serum is still maintained: that is, water and glucose cross the cell membrane in equal proportions, the transfer of water being conditioned by the transfer of glucose. It should be mentioned that the conditions under which glucose is not evenly distributed in human blood are not encountered in life.

With this much established it became necessary to learn whether red blood cells behave in the same manner while they are in circulation in the body. With this end in view Eisenman and Hald[5] examined blood of patients before, and at intervals after, injections of large quantities of hypertonic sodium chloride or sodium sulfate. In these experiments (see Table II) the base of serum increased from 6 to 27 millimols between the two observations, which were made, in two instances, at intervals of as much as eighteen hours. In every experiment except the ones surrounded by the black line, in which a single analysis was probably in error, the ratios of cell water and of serum base agree quite as well as they did in the *in vitro* experiments. It would seem, then, that both in the test tube and in the body the red blood cells respond to changes in the concentration of base in the serum as if they were quite impermeable to base, adjusting osmotic equilibrium by transfers of water alone. As far as their size and water content are concerned, then, these cells would seem to be entirely at the mercy of the serum in which they are bathed.

In early experiments *in vitro*, in which whole blood and serum were investigated by Wakeman and Eisenman[4] it was also proved by direct analysis that no base passed into or out of the cells. This has since been confirmed with more exact techniques by Eisenman and Hald.[5] To our great concern however, when the same techniques were applied to the *in vivo* experiments unmistakable changes in the base of cells were detected. The two series of experiments are contrasted in Table III. The calculated limit of error in the estimation of $Bc_2 - Bc_1$, is 4 millimols, an

TABLE II

Transfers of cell water in relation to changes of serum base.

Experiment	*(1)* $\frac{W_{c2}}{W_{c1}}$	*(2)* $\frac{B_{s1}}{B_{s2}}$	*(1)* / *(2)*
M	89.3	94.7	0.94
T 1-2*	100.0	83.6	1.20
1-3*	96.6	85.9	1.12
2-3	104.7	102.7	1.02
Bo	93.3	96.2	0.97
Be	96.0	97.0	0.97
Cr	93.2	95.8	0.96
Ch	95.3	96.2	0.99
S	94.2	94.8	0.99
Tr	98.7	94.3	1.05
		Average *	0.99

* Experiments T 1-2 and T 1-3 are excluded

TABLE III

Transfers of base between cells and serum

	In vivo				*In vitro*		
Exp.	*(1)* B_{c1}	*(2)* B_{c2}	*(2)-(1)*	*Exp.*	*(1)* B_{c1}	*(2)* B_{c2}	*(2)-(1)*
M	97.4	101.3	3.9	1	100.8	101.3	0.5
T 2-3	96.6	87.7	-8.9	2	101.3	105.3	4.0
Bo	98.6	86.3	-12.3	3	117.8	116.7	-1.1
Be	111.7	105.1	-6.6	4	112.3	110.4	-1.9
Cr	101.4	113.5	12.1	5	112.8	113.7	0.9
Ch	107.6	102.2	-5.4				
S	104.9	93.4	-11.5				
Tr	106.6	107.5	0.9				

estimation that seems to be supported by the *in vitro* experiments. Nevertheless this was exceeded in five of the eight *in vivo* experiments, the very experiments in which changes of cell water and analyses of serum gave no evidence of transfer of base. A paradox therefore presented itself.

To find the source of this paradox it is necessary to turn to more direct consideration of the composition of the cells. By reference to Fig. 1 which compares the average molar concentrations of bases per unit of water in serum and cells, it may be seen that there is slightly more total base in serum. In addition the base of serum is overwhelmingly composed of sodium, while in the cells potassium predominates. The concentrations of total base in the two media are, on the whole, sufficiently alike, to

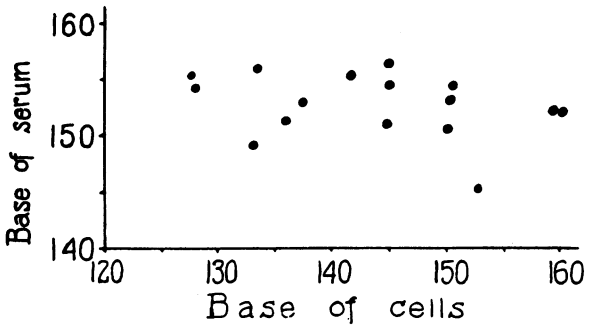

Fig. 3—Comparison of base in water of cells and serum of human blood.

give the impression that, in the cells as in serum, inorganic salts are chiefly responsible for the osmotic pressure. Since the osmotic pressure of both media is the same, the concentrations of base in cells and serum should vary directly. That this is not the case is quite evident from Fig. 3, in which the base of serum is plotted against the base of cells. It is obvious that serum base varies within relatively narrow limits, cell base varies over a much larger range. Furthermore there is no demonstrable relation between the two. At one end of the scale the ratio of cell base to serum base is 0.86, at the other end, 1.05. If these figures are to be accepted potassium and sodium cannot have as important an osmotic role in cells as they do in serum. Osmotic equilibrium can exist when the concentration of base is distinctly higher in the cells than in the serum. In this case, presumably, a fraction of the base in the cells must be osmotically inactive (perhaps because it exists in non-ionized form) or else the serum must contain some unusual unrecognized components. The latter is the less probable explanation, since the composition of serum is simpler and more uniform than that of cells. In those instances where base is far lower in cells than serum, it must be supposed that other chemical substances in cells are bearing more than their usual share of the osmotic load. Regardless of the explanation, unless the analyses are incorrect—and this possibility has been abandoned after exhaustive examination of the methods—it appears that any variation of base in serum is attended by a proportional change of osmotic pressure; whereas the base of cells can vary within wide limits without affecting osmotic pressure.

At first sight this conclusion seems absurd because it is so much at variance with traditional teaching. Emphasis has, in the past, been placed

on the average concentrations of base in cells and serum which, as you saw in the slide a short while back, are nearly the same. Variations in individual bloods were attributed to errors. It was to eliminate this excuse that Miss Hald[7] perfected the techniques by which these analyses were made. On the face of it the results before you are less absurd chemically and biologically than the old concepts. In serum salts make up the major portion of the solutes, and in such a simple medium, may be expected to be quite completely ionized and active. Osmotic comparisons with salt solutions and chemical comparisons with natural and artificial transudates, moreover, have demonstrated that serum meets these expectations. The contents of the cells are far more complex and contain many substances which do not exist in serum, but which must contribute to the osmotic pressure. Moreover, in these cells metabolic processes are causing continuous permutations and combinations of solutes. Complex compounds are breaking down and being again reconstituted in a perpetual cycle. Were there not some mechanism by which these reactions could be, so to speak, osmotically buffered, they would produce constant—sometimes large—changes in volume and water content of the cells. How, if these occur, have they escaped observation? There is adequate precedent for variation of the osmotic activity of base in the combinations of calcium with protein in serum. There must, in addition, be some means by which cells can, in times of need, accumulate stores of material without being forced to take on at the same time excessive amounts of water. The aggregation of glucose into large molecules of glycogen is an example of a reaction that must serve just this purpose. Finally, this cell can not be so utterly impervious to base as our osmotic experiments would indicate or it would be forever deprived of some of the elements that are essential for its vital activities. It remains, then, to discover the mysteries of the facultative permeability of the cell.

In an attempt to learn the significance of the changes of serum phosphate that accompany rapid movements of glucose in the body, it seemed highly advantageous to use whole blood for analyses rather than serum. It had been rather generally assumed that the red blood cell membrane was freely permeable to inorganic phosphate. When Dr. Lena Halpern[8] investigated the subject, this proved not to be the case. The distribution of inorganic phosphate between cells and serum is quite capricious and does not change when potassium and sodium phosphates are added to blood.

And now I should like to make a slight diversion. In all the *in vitro* experiments on permeability that have been mentioned special efforts were made to prevent intracellular activities. Blood was treated anaerobically and kept cool. If exchange of gases is prevented blood can be kept at ordinary refrigerator temperatures for from twelve to twenty-four hours without appreciable change, at room temperature for from two to four hours. If it is warmed to body temperature glucose in the cells is oxidized, organic phosphate esters are broken down to inorganic phosphate and lactic acid is formed. These reactions can be modified by exposure to oxygen or CO_2 and can be reversed by the addition of glucose. Dr. Halpern found that during the glycolytic process inorganic phosphate passed out of the red cells into serum. The process by which it escaped was not merely diffusion, since phosphate was transferred to serum even when its concentration in the latter had been greatly increased by the addition of inorganic phosphate. The escape of phosphate did not denote injury of the cells because the direction of the flow of phosphate could be reversed by the addition of the glucose. It remains only to add that Hald found that potassium accompanied phosphate across the membrane. It can be inferred from these experiments that the red blood cell is always in osmotic equilibrium with its environmental fluid, the blood serum, and responds by changes of volume and exchanges of water to alterations of the concentration of base in serum. As long as it is in the resting state, moreover, its membrane remains impermeable to the bases, to phosphate, to sulfate; but during metabolic activity base and phosphate pass in one direction or another, presumably in accordance with the needs of the cell. These transfers are not accomplished by a mere process of diffusion and must therefore involve chemical reactions.

To demonstrate such exchanges in the body and to measure their osmotic effects has proved more difficult. In the test tube glycolytic processes are accompanied by changes of blood pH, CO_2 and oxygen that confuse the picture greatly. In life it has proved hard to induce transfers of phosphate of sufficient magnitude to cause measurable changes of osmotic pressure. After administration of insulin and glucose, in a few instances, relatively large shifts of phosphate have been demonstrated by Miss Hald and Dr. Margaret Dann.[9] With some reservation it may be stated that these shifts could not be correlated in direction or magnitude with the slight transfers of water which sometimes occurred. This is suggestive, but by no means conclusive, evidence that the phos-

phate and base which were transferred had little or no osmotic activity.

So much time has been wasted on this red blood cell, not because of its intrinsic importance, but because it is peculiarly adapted to such investigations. Throughout it has been recognized that it might prove quite different in its reactions from other more active cells, and therefore a useless model. However, there was continually growing evidence that some of its characteristics were shared by muscle and other tissue cells. These I shall not attempt to treat historically. It has been generally assumed that between the active cells of the body and the blood vessels there is a body of fluid, the interstitial fluid, that has the composition of an ultrafiltrate of serum. Our knowledge of this fluid is entirely based on inference and information obtained in pathological conditions accompanied by transudation. In the latter it must be assumed that the transudates are merely expansions of an already existent body of fluid. If there is interstitial fluid it should have the composition of an ultrafiltrate of serum, being chiefly composed, so far as inorganic elements are concerned, of sodium and chloride. Analyses of muscle tissue by Fenn,[10] the Eggletons,[11] Eichelberger and Hastings[12, 13, 14] and others have proved that the proportions of chloride and sodium in muscle are much the same as those in serum and that the total quantities of these substances in muscle can be accounted for if about 20 per cent of the muscle mass consists of interstitial fluid. Furthermore, if isolated, living, resting muscle is immersed in sodium chloride solutions the salt apparently diffuses into about the same volume of the muscle. Studies by Eggleton[15] indicate that inorganic phosphate, also, diffuses into about the same proportion of the muscle mass. Urea, on the other hand, diffuses freely throughout all the water of the muscle.[16] From these experiments it would appear that there is a barrier to the free diffusion of some solutes through muscle which distinguishes about one fifth of the tissue from the other four fifths. Harrison, Darrow and Yannet[17] analyzed whole animals for chloride, sodium and potassium. They found that, if bone were excluded, the total quantities of sodium and chloride in the bodies of dogs, monkeys and rabbits could be accounted for if 20 to 30 per cent of the body were composed of an ultrafiltrate of serum; leaving enough potassium and magnesium to supply base for the remainder of the water in the body. It seems not unreasonable to believe, therefore, that there is extracellular fluid amounting to about one-fifth of the body weight, and that the remainder of the water in the body, within the cells, is almost, if not quite,

devoid of sodium and chloride. Eichelberger and Hastings[12, 13, 14] have shown that this hypothesis affords an adequate explanation for the changes in the composition of muscle that are produced by injections of normal salt solution, by alkalosis and acidosis and by various dehydrating measures which alter the osmotic pressure of the serum and, therefore, cause the muscle cells to swell or shrink.

To make the analogy to the red blood cell still closer, Pollack, Flock, Mason, Essex and Bollman[18] perfused isolated hind limbs of dogs with blood to which they added inorganic phosphate. Analyses of the muscles showed that the inorganic phosphate did not diffuse into the cells; nevertheless, when glucose and insulin were added to the perfusate the concentration of phosphate esters in the muscles increased. Presumably, then, under the stimulus of metabolic activities chemical processes were activated which conveyed phosphate across a cell membrane which it did not ordinarily traverse. Fenn and Cobb[19] and Thaler[20] have found that potassium can be made to escape from muscle by various measures such as exercise and hemorrhage. Fenn has concluded that the membrane is always permeable to base, but not to anions, and attributes these exchanges to alterations of pH, which have not always been demonstrated.[20] It seems quite as possible that they are expressions of metabolic activities within the cells.

The application of these principles to the analysis of phenomena of human physiology and pathology in a truly quantitative sense begins with Gamble's[21] classical studies of the salt metabolism in fasting and acidosis. Although I shall not dwell on these in detail I cannot refrain from paying him tribute for the inspiration he has given to all those who have worked on problems of salt and water metabolism. Gamble recognized the importance of distinguishing between extracellular and intracellular losses of water and salt and demonstrated the disasters that come from reducing the osmotic pressure of the body fluids by depleting them of salt. He also demonstrated the tenacity with which osmotic pressure is maintained in the face of metabolic disorders. He pointed out the intracellular segregation of potassium and the extracellular distribution of sodium and chloride, and the possibility of distinguishing by balance studies the source of losses of cations and water. It was not, however, possible by the technique which he employed to allocate these cations with accuracy nor to measure directly the relative changes in volume of interstitial and cellular fluids. The chief difficulty in this respect lay in

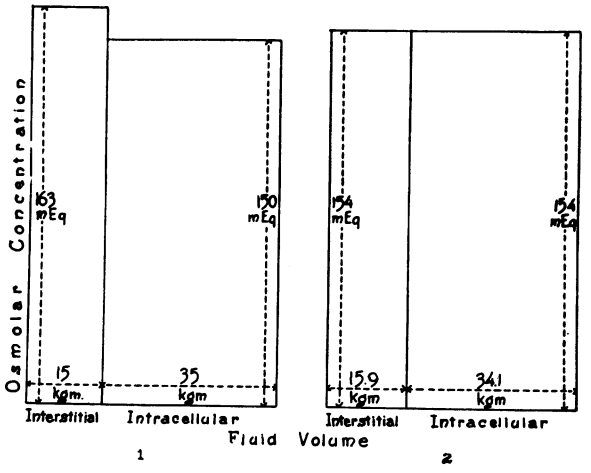

Fig. 4—The effect of adding salt to interstitial fluid.

the fact that, even if sodium is entirely confined to the extracellular fluids, changes in its concentration, because they alter osmotic pressure, will disturb the distribution of water between cells and interstitial fluid. This is illustrated in Fig. 4. In the first part are shown two solutions, each containing base in a concentration of 150 millimols per liter, separated by a membrane which is pervious only to water. If enough salt is added to the smaller compartment to increase the osmolar concentration by 13 millimols, water will enter from the larger compartment until the osmolar concentration is again equalized. At this point, pictured in the second part of the figure, it will be found that, since the ultimate osmolar increment in both compartments is identical, the effect on the concentration of salt in either compartment will be the same as if the salt had diffused freely through the membrane. The volumes of the two compartments have changed; but those changes of volume cannot be detected by the usual methods of chemical examination which measure concentration only.

Theoretically this difficulty could be circumvented by a simple expedient. If, instead of one substance, two substances were used, which originally existed in plasma in different concentrations, it should be possible by simultaneous equations to determine how far they were diluted by water derived from the cells or concentrated by passage of water into the cells. The two substances chosen were sodium and chloride. I shall not tire you now with the details of the experiments which have been published by Lavietes, D'Esopo and Harrison.[22] Suffice it to say

that the concentrations of chloride and sodium did not differ enough to permit accurate deductions from clinically practicable procedures in any but extreme cases. The results were, however, sufficiently compatible with our premises to stimulate new endeavors.

Some method of measuring the interstitial fluids was obviously needed. Substances must be found which were not normal constituents of serum and which would pass freely through vascular walls without penetrating cells. To make a long story short, after searching the literature and considering the adventures with red blood cells, three substances were selected that seemed likely to meet requirements: sulfocyanate, which Crandall and Anderson[23] had shown diffused through about 20 per cent of the body; sulfate and sucrose, neither of which penetrated the red blood cell. All these substances can be quantitatively recovered in the urine, proving that they are not subjected to chemical or metabolic changes. For various reasons none proved quite ideal for the purpose for which it was intended. Sulfocyanate enters red blood cells and apparently combines to some extent with the lipoids of blood plasma; sulfate and sucrose are excreted too rapidly by the normal kidney. In addition sulfate figures must be corrected for endogenous serum sulfate as well as endogenous sulfate excretion. Nevertheless some encouraging measurements were made by Lavietes, Bourdillon and Klinghoffer.[24] The principle of the methods was quite simple. The test material was injected (or, in the case of sulfocyanate, sometimes ingested). After an appropriate interval samples of blood and urine were collected and analyzed for the test substance. The total amount injected minus the quantity excreted, divided by the concentration in the water of serum, will give the volume of fluid in which the material is dissolved if it is distributed through this field in a uniform manner. Results of certain comparisons of the distribution of the three test substances are given in Table IV. When the objections to the three substances are considered, the agreement appears highly satisfactory. It is hard to escape the conviction that there is a barrier which separates the fluids of the body in a broad way into two compartments, of which one, into which these substances chiefly or solely diffuse, makes up roughly 20 to 30 per cent of the body weight, for that is what these figures imply in terms of the total body mass.

An attempt has been made more recently by Purple and Lavietes[25] to utilize the same principle for the measurement of the total water of the body. For this purpose urea, because of its known universal diffusibility,

TABLE IV

The volumes of distribution of sucrose, sulfocyanate and sulfate in the body.

Subject	*Volume of distribution of*		
	Sucrose liters	*CNS liters*	*SO_4 liters*
1	18.8	18.2	
			18.7
	18.3	18.4	
2	18.3	19.0	
3	15.3	17.4	
4		16.6	
			18.8
		17.0	

seemed the most suitable test substance. However, because of the amounts of endogenous urea in the blood and the variability of endogenous urea formation and excretion and the diuretic effect of urea, it proved disappointing. In its place thio-urea was chosen. This compound possesses most of the chemical and physiological properties of urea, and is excreted completely, unchanged, in the urine. It might be considered as a labelled urea molecule. Unfortunately specific quantitative chemical methods for its detection and measurement are not highly sensitive, therefore large quantities of the compound had to be given, so large as to cause serious gastric symptoms. However, before the connection of these symptoms with the drug was established, measurements of total fluids were made on two normal persons and one patient. The volume of distribution varied from 68 to 70 per cent of the body weight, values consistent with accepted conceptions of the water content of the body. Attempts are now being made to refine the analytical technique so that the method may be made clinically feasible.

Meanwhile, since the experiments with sulfocyanate, sucrose and sulfate gave reason to believe that sulfate did not enter cells, it occurred to Bourdillon and Lavietes[26] that the distribution of sodium could be traced more accurately if it were injected in the form of sodium sulfate, earmarking the exogenous sodium ions, so to speak. Large quantities of hypertonic sodium sulfate were, therefore, injected into normal subjects. Blood serum before and after the injections was analyzed for sulfate, chloride, bicarbonate, base or sodium, and protein. In addition oxygen capacity and cell volume of whole blood were measured, and urine was analyzed for potassium, sodium, chloride and sulfate. Finally sodium sul-

TABLE V

Intravenous injection of 102 m.eq. of Na_2SO_4 and 250 cc. H_2O

	1	2	2-1	2/1
Serum SO_4	0.5	15.3	+14.8	
B	148.8	154.5	+5.7	
Cl	98.5	93.1	—5.4	105.8
Distribution volume of				
CNS	16.2	17.1	+0.9	105.5
SO_4	—	12.9		
Change of B estimated from Cl		140.5	+14.0	
Interstitial fluid change from B and SO_4				106.5

focyanate was given in advance of the experiments to serve as a check upon the volume of the interstitial fluid. Time was not given for the sulfate to become completely distributed because this would have permitted the excretion of too much of the salt. The results of one such experiment are shown in Table V. The first column represents conditions before the injection; the second column after the injection; the third column the changes in the blood as the result of the injection; and the last column the ratio of the final volume of the interstitial fluid to the initial volume, estimated by a variety of methods. The quantities of sodium, sulfate and water retained are calculated from the amounts given, corrected for the quantities excreted in the urine. At first sight it might be anticipated that, since equal quantities of the two ions were injected and equal quantities were excreted, the concentrations of base and sulfate in serum would increase to the same extent. Actually sodium rose only 5.7 milliequivalents, while sulfate rose 14.8 milliequivalents. If however, attention is turned to chloride, it is seen that this fell 5.4 milliequivalents, although negligible amounts of chloride appeared in the urine. It would appear, then, that the body of fluid containing chloride was diluted to the extent shown in the last column, 5.8 per cent. Comparison of the sulfocyanate figures indicates that the volume of the interstitial fluid increased from 16.2 to 17.1 liters, a difference of 0.9 liters or 5.5 per cent. If it be assumed that sodium was restricted to the same body of fluid, the sodium originally in this fluid must have been diluted just as much as the chloride. At the end of the injection it should have fallen from its original concentration of 148.8 to 140.5. If this value, which is placed between the first two columns, is subtracted from the final figure 154.5

the actual increment of exogenous sodium is found to be 14.0 milli-equivalents, an extremely satisfactory agreement with the 14.8 milli-equivalent increment of sulfate. If it be assumed that the increments of base and sulfate were identical it can be estimated from the changes of sulfate and sodium that the interstitial fluids expanded 6.5 per cent. The data are, then, entirely compatible with the theory that sulfate, chloride, sodium and sulfocyanate were distributed over an identical fraction of the total water in the body and that this fraction, approximately one twentieth of the body mass, increased in volume by about 1 liter. If it did expand to this extent some of the extra water must have been acquired from other parts of the body, because the volume added by the infusion, when corrected for that excreted in the urine, amounted to only about 200 cc. The simplest explanation of the facts, in view of other evidence that has been adduced, would seem to be that the sodium sulfate entered only the extracellular fluids. Because it increased the osmotic pressure of these fluids water was withdrawn from the cells to restore osmotic equilibrium. It is possible to calculate that, if this was the case, the cellular fluid volume was about twice as large as the extracellular. This experiment is one of those in which it was earlier shown that the red cells contracted in proportion to the increase of base in the serum, about 4 per cent. The estimated contraction of the tissue cells falls somewhat short of this, which is to be expected since comparison of sulfate and sulfocyanate distributions in column 2 shows that the adjustments occasioned by the injections of salt had not yet come to equilibrium. This is only one of the most complete of a consistent series of experiments by Bourdillon and Lavietes.[26]

In general, then, tissue cells appear to resemble red blood cells in their behavior. There is some evidence that sodium may be more completely excluded from the tissue cells and that these cells are almost, if not quite, devoid of chloride. Furthermore, these two ions are effectively excluded from the cells. When the concentration of sodium in the interstitial fluid changes, osmotic equilibrium is restored by transfers of water to or from the cells without transfers of base. No evidence has been adduced that base is admitted to or discharged from the cells in behalf of osmotic equilibrium *per se*. If these inferences are justified the maintenance of the osmotic integrity of the interstitial fluids should be of prime importance to the function of the cells, which must imbibe water and swell every time the concentration of sodium in the serum falls, or shrivel

whenever it rises. The concentration of sodium in the interstitial fluids controls, however, only the load of water in the cells, that is the dilution of the cellular constituents. The actual quantities of these constituents in the cells—or at least the inorganic components—appear to be controlled by entirely different mechanisms. The impermeability of the resting cell to potassium, phosphate and other chemical substances is an essential protective property. It is equally essential that there be some means to unlock the barrier when need arises in the cells for more of these materials or for the discharge of any excess. Apparently the key to the barrier is entrusted to the metabolic processes of the cells, thus insuring automaticity.

Although potassium is the predominant base of all cells, including the red blood cell, it is quite as impossible to drive potassium as it is to drive sodium across the membrane of the resting red blood cell. This may not be true of other cells. Bourdillon[27] has found that when potassium chloride is given by mouth, the potassium distributes itself through a larger volume of fluid than the chloride does, a volume that approaches the total amount of fluid in the body. It seemed possible that the potassium might be absorbed from the gut more slowly or less completely than the chloride. But, when Winkler (unpublished studies) gave potassium chloride intravenously he obtained similar results. The increments of serum potassium and chloride in these experiments, which were conducted on human subjects, are small; but the differences in the increments are consistent and unmistakably significant. Moreover, in similar experiments Bourdillon found that, after sodium chloride, increments of sodium and chloride were the same. These experiments, if they are substantiated by further work, can only mean that inorganic potassium, when it gains access to the interstitial fluids, passes freely into the cells. Since exogenous potassium is excreted quite rapidly, it must escape from the cells with the same ease. The red blood cell model at this point fails. Of course the potassium which cannot be found in the interstitial fluids may seek other repositories than the cells. To jump to this conclusion at once is quite unjustifiable. It is equally unjustifiable to assume that the mere increase of potassium in the interstitial fluid initiates some metabolic disturbance by which it is conveyed into the cell. Since the volume of distribution of the potassium approaches the total fluid of the body, the simplest hypothesis is that inorganic potassium diffuses freely across the cell membrane. In this case, the intrinsic potassium of cells, since it does not diffuse

outward, must be restrained from free diffusion by some force other than the mere impermeability of the envelope of the cell.

This would not be incompatible with any of the facts thus far presented. So long as sodium is ionized and cannot gain access to the cells, changes of its concentration must control the size and water content of the cells. If inorganic potassium can diffuse freely between cells and interstitial fluid it would have no influence upon the distribution of water. This fraction of potassium is quite small. The great mass of potassium may be relatively non-ionized, combined with organic phosphate esters and protein. Presumably this would be in equilibrium with the moiety of ionized inorganic potassium and, therefore, not entirely unaffected by the concentration of the latter. Its concentration would, however, depend far more upon the material available for the formation of the compounds in which it is found, of which phosphate is presumably one of the most important components. Since phosphate can enter or leave the cell only under the stimulus of metabolic processes, movements of potassium would be conditioned by these same metabolic processes which control phosphate. All this discussion of potassium is, of course, highly speculative in comparison with the preceding discussion of sodium, which is experimentally documented. It is, however, consonant with the physiologic and pathologic data now available.

Gamble[21] found that in the dehydration of starvation acidosis potassium as well as sodium was wasted. This he connected first with losses of protein and glycogen from the cells. Later[28, 29] he discovered that potassium was sacrificed also in the diuresis evoked by acidifying salts, this time without appreciable losses of carbohydrate or protein. It seemed possible that wastage of extracellular sodium might itself provoke the discharge of potassium from the cells and that this might serve to mitigate the effects on the cells of altering the amount of salt in their environment. Nevertheless, in experiments by McCance[30] in which the sodium of the body was greatly depleted by withdrawal of salt from the diet combined with sweating, potassium losses were quite insignificant. In these experiments it was estimated that at least 25 to 30 per cent of the sodium and chloride in the body was withdrawn and the concentrations of these substances in the serum fell 10 to 15 millimols.

In Addison's disease and in some animals after adrenalectomy sodium wastage is accompanied by retention of potassium. This retention, according to the balance studies of Harrop and his associates,[31] causes no

immediate rise of serum potassium. The potassium which is retained apparently enters, or becomes imprisoned in, the cells. Harrison and Darrow,[32] by analyses of the tissues of rats, animals which do not waste sodium, have shown that after adrenalectomy cellular potassium increases without a comparable increase of water. If this is verified, it exemplifies the accumulation in the cells of osmotically inactive potassium. It also suggests that removal of the adrenals gives rise to a widespread derangement of cellular metabolic processes. It has been demonstrated further that adrenalectomized dogs are benefited by restriction of potassium in the diet and can be thrown into shock by the administration of potassium salts.[33] This is consistent with the hypothesis that the intrinsic potassium of the cells is affected by the supply of inorganic potassium in the interstitial fluid. These discoveries of the role of potassium detract in no respect from Loeb's[34,35] discovery that in man and many animals absence of the adrenal cortex causes depletion of sodium and symptoms that can be relieved in whole or in part by administration of sodium salts. Harrison and Darrow's rats, in spite of the fact that they did not waste sodium spontaneously, proved more susceptible than normal rats to withdrawal of sodium. Moreover, when the sodium in their interstitial fluids fell, the tissue cells swelled, proving that they had lost none of their capacity to respond osmotically.

A recent unpublished study by Klinghoffer and Lavietes of a patient with Addison's disease may be reported with a certain amount of reserve. He proved to be on the verge of a crisis and, by the time the three-day study was completed, required cortical extract to revive him. Throughout the whole period of observation, therefore, he was sinking deeper into shock in spite of vigorous administration of saline. From Table VI it can be seen that the concentration of sodium in the serum was only slightly reduced and potassium was normal as were also chloride—and bicarbonate which does not appear in the table. Moreover, because of the vigorous therapy they did not change appreciably. There was a large positive balance of sodium and chloride and a smaller negative balance of potassium. During this period (see Table VII) the volume of the interstitial fluid, measured by sulfocyanate, rose from 20.0 to 24.7 kgm., an increase of 4.7 kgm. Estimations by means of the sodium and chloride balances indicated increases of 3.2 and 3.8 kgm. respectively. The agreement is not perfect, but satisfactory enough under the circumstances. It seems reasonable to believe that the volume of the interstitial fluid in-

TABLE VI

Sodium and potassium balances in a case of Addison's disease.

Composition of serum water

m. eq.	*June 22*	*June 25*	*Change*
Sodium	141.3	140.5	—0.8
Potassium	5.1	4.7	—0.4
Na + K	146.4	145.2	—1.2

m. eq.	*Sodium*	*Chloride*	*Potassium*
Intake	1851	1659	113
Output	1395	1255	227
Balance	456	404	—114

TABLE VII

Changes in distribution of water in a case of Addison's disease

Interstitial fluid

kilos	*June 22*	*June 25*	*change*
from SCN	20.0	24.7	+4.7
from Na	—	—	+3.2
from Cl	—	—	+3.8
Conservative estimate			+3.5
Weight	73.0	74.5	+1.5
Fluid received from cells			+2.0

	m. eq.
Cellular base loss expected	250 to 300
Potassium loss observed	100
Accumulation of cellular K	150 to 200

creased 3 or 4 kgm. At the same time the patient's weight increased only 1.5 kgm. Therefore the interstitial fluid received about 2 kgm. of water from the cells. But with this 2 kgm. of water only about 100 milli-equivalents of potassium were lost, a far smaller amount of potassium than even the most conservative estimates would credit to this quantity of cellular water. This suggests that potassium became more concentrated in the cells. Analyses of blood (Table VIII) showed that the concentrations of potassium and potassium + sodium in the red blood cells were far higher during this crisis than they were a month or two later when

TABLE VIII

Changes in the composition of serum and cells in the blood of a patient with Addison's disease.

Milliequivalents per kilo of water

	June 22	*June* 25	*July* 31	*August* 12
Serum				
Na	141.3	140.5	142.3	143.2
K	5.1	4.7	3.8	5.0
Na + K	146.4	145.2	146.1	148.2
Cell				
Na	20.7	31.2	27.3	15.7
K	142.5	127.2	98.2	107.6
Na + K	163.2	158.4	125.5	123.3

the patient was in excellent condition. Too much emphasis should not be placed upon these quantitations, especially those that deal with the concentrations of base in the red blood cells, because just before the patient died a few days ago in another crisis, the potassium and sodium of his red blood cells had not risen again. However, on this occasion sodium of the serum was distinctly lower. It may be of some significance that in the balance studies water and electrolytes moved in the directions that the experiments of Harrison and Darrow would lead one to anticipate.

In another series of experiments Yannet and Darrow[36] have shown that hyperthermia causes similar osmotically inactive accumulations of potassium in cells; but in this case the changes are restricted to the central nervous system.

Depletion of serum sodium is extremely common in clinical medicine. It is regularly encountered in the states of dehydration which result from severe vomiting, diarrhea or gastro-intestinal fistulae; in diabetic acidosis; in water intoxication; in lobar pneumonia; in terminal stages of nephritis; and sometimes with no discoverable cause. It has been too generally assumed that in all these conditions as well as Addison's disease depletion of base has the same quantitative and qualitative effects. Nevertheless, those who have compared, in any large series of patients, the concentrations of electrolytes in the serum with the physical condition, must have been impressed with the wide range of symptomatology associated with deficits of base and with the variability of the response to restoration of the concentration of salt in the serum. The dramatic condition of the

patient in the crisis of Addison's disease differs strikingly from that of the person who has lost sodium by the vomiting of pyloric stenosis, diarrhea or diabetic acidosis. Mere replacement of sodium and water in a severe crisis of Addison's disease may be little more than a futile gesture; I suspect that the administration of cortical extract in pyloric stenosis or diabetic acidosis would be equally fruitless.

In advanced nephritis the sodium of serum may become greatly reduced. Frequently such salt deficits are attended by disturbances so profound that the condition has been named "uremia from lack of salt". If the advanced nephritic is given a salt poor diet, especially if large amounts of fluid are also administered, both base and chloride of the serum will fall and the weight will usually diminish. Appetite and thirst may fail and the blood non-protein nitrogen often rises even if no more serious symptoms develop. We have reported[37] a nephritic who displayed this tendency to waste sodium to such an extent that when he was given as much as 15 gm. of sodium chloride daily, the chloride of the serum rose only to the upper normal limit, 105 m.eq. When salt was withdrawn it fell gradually to 85 m.eq., at which point the total base of the serum was only 125 m.eq. That is, both sodium and chloride were almost 20 m.eq. below normal, reductions quite as great as those encountered in the crises of Addison's disease. In spite of this extreme salt depletion and the hypotonicity of his serum the patient felt none the worse and exhibited none of the symptoms which commonly accompany such salt deficits. The red blood cells in this case swelled in direct proportion to the reduction of base, and from studies of the electrolyte balance it may be inferred that the tissue cells behaved similarly.[22] Freedom from symptoms cannot be attributed to any peculiar compensatory reaction which protected his tissues from the reduction of osmotic pressure. One peculiar feature was noted: Thirst did not flag when salt was withdrawn and the body weight varied only 2 kgm. in the course of the experiment. He did not suffer the dehydration which usually follows salt restriction in advanced nephritis.

Winkler and Crankshaw[38] have recently reported a series of patients with advanced pulmonary disease, especially tuberculosis, who exhibited a peculiar tendency to waste sodium and chloride in the urine and who had persistently low concentrations of these substances in the serum. The conditions could easily have been confused with Addison's disease except that salt depletion in these cases had no spectacular consequences.

Further examples of the variable effects of salt deficits could be mul-

tiplied to no great advantage. If I should dare to differ from Gamble in any important respect it would be to challenge his statement that the concentration of base in the interstitial fluids is more jealously guarded than is the volume of these fluids.[39] Unfortunately this doctrine is one to which I so long adhered that it ill befits me to do more than confess my own error. Apparently, in this as in other matters, circumstances alter cases and it is incumbent upon us to inquire into the circumstances. The determination of concentrations of salts in the serum is a unidimensional measurement. This must be supplemented by measurements of the volumes of the fluids in which those salts are dissolved if precise information is to be secured about animals which have three-dimensional bodies. Even this does not suffice: the allocation of these fluids and solutes between the various compartments of the body cannot be neglected. Among these compartments the blood stream, the interstitial fluids and the cells must be separately considered. Although the blood serum is only part of the interstitial fluid, the shock of hemorrhage and the edema of heart disease or nephritis can leave no doubt that the distribution of fluid between the subdivisions of this general system is of great moment. Harvey's and Starling's circulations cannot be forgotten. Mere expansion of the interstitial fluid as a whole seems to cause little disturbance; but considerable reduction of its volume, even without change of composition, is not so well tolerated. Changes of its osmotic pressure (i.e., the sodium concentration) usually, but not invariably, provoke serious symptoms. Why there should be exceptions remains to be discovered. Are the untoward effects of base depletion entirely referable to changes in the volumes of the cells as has been suggested? How large a part must be attributed to the concomitant loss of interstitial fluid with which base depletion is usually associated? Do those who apparently escape evil effects escape because the volume of the circulating blood or the interstitial fluid remains relatively unaltered, or by virtue of some compensatory transfer of base or by the inactivation of previously active osmotic components of the cells? Finally, what are the processes that unlock the cellular membranes and possibly alter the osmotic activity of the cellular constituents and how can they be controlled?

It is halting first attempts at dynamic measurements of the distribution of fluids and salts and the forces that govern them which I have tried to describe tonight. Tentative inferences have been drawn of which it is hoped that some at least may survive the acid test of further investigation.

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